Nuclease

Week 14

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

Summary

1 Addition of BamHI restriction site downstream to the nuclease sequence and amplification of the whole plasmid, using overhang PCR

Responsible

Oscar He and Reskandi Rudjito

Protocols used

PCR Amplification (Phusion)
Gel electrophoresis

Modifications and comments to protocols

PCR Amplification (Phusion) primers:	Nuc_R (reverse) Suffix_F (forward)
PCR Amplification (Phusion) buffer:	HF Buffer

Experimental Set Up

Table 1: Concentration of samples used for the ovarhang PCR amplification.

Sample	Concentration [ng/μl]
pSB1C3-T7-Nuc (diluted from 288.6 ng/μl)	10

Table 2: Volumes of components in PCR mix.

Component	Volume μl]
10 μM Suffix_F	1
10 μM Nuc_R	1
5X Phusion HF Buffer	4
Phusion DNA Polymerase	0.2
2 mM dNTP mix	2
Template DNA	1
ddH_2O	10.8

Table 3: Thermocycling conditions, with two different annealing temperatures.

Step	Temperature [°C]	Time [sec]
Initial Denaturation	98	30
30 x	98	10
	55 (57)	30
	72	60
Final Extension	72	600
Hold	4	N/A

Results and Conclusions

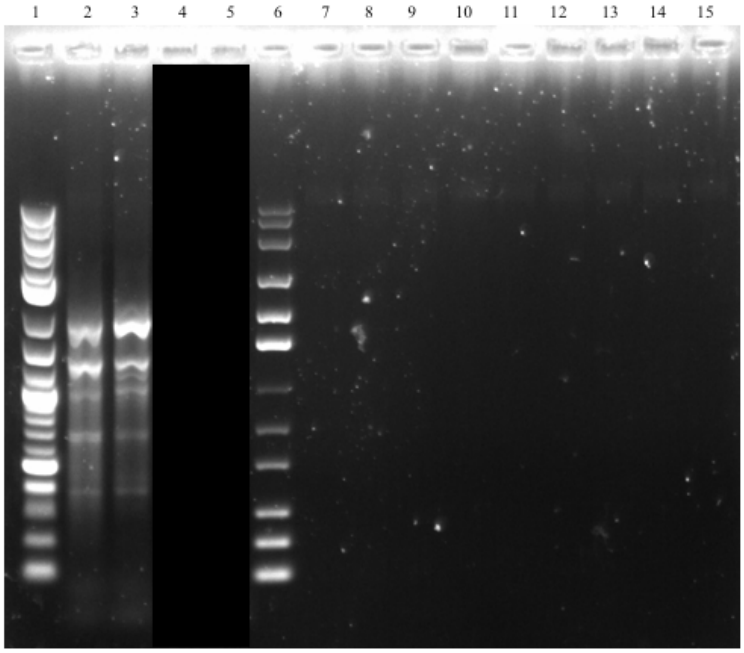

Figure 1: Result of the gel electrophoresis of overhang PCR product, with 1 % agarose at 110 V. (1) DNA Ladder (2) Nuc 55 °C (3) Nuc 57 °(6) DNA Ladder.

Discussion and Troubleshooting

Since this was the first time using the Phusion polymerase the different parameters set might not have been the optimal. For all four samples, the actual bands have smaller sizes than expected (approximately 2672 bp for all). This is believed to be due to a too short extension time. The annealing temperature 57 °C showed a better amplification than 55 °C, especially when analysing the lysostaphin samples, which were run in the PCR. For next overhang PCR of the whole plasmid with Phusion DNA polymerase, the annealing temperature and the extension time will be increased. After discussion with our advisor, Kristina Westerlund, GC Buffer will be used instead of HF Buffer.

2 Addition of BamHI restriction site downstream to the nuclease sequence and amplification of the whole plasmid, using overhang PCR - second attempt, with PCR purification

Responsible

Oscar He, Reskandi Rudjito, and Ellinor Lindholm

Protocols used

PCR Amplification (Phusion)
PCR Purification
Gel electrophoresis

Modifications and comments to protocols

PCR Amplification (Phusion) primers:	Nuc_R (reverse) Suffix_F (forward)
PCR Amplification (Phusion) buffer:	GC Buffer

Experimental Set Up

Table 4: Concentration of samples used for the ovarhang PCR amplification.

Sample	Concentration [ng/μl]
pSB1C3-T7-Nuc (diluted from 288.6 ng/μl)	10

Table 5: Volumes of components in PCR mix.

Component	Volume [μl]
10 μM Suffix_F	1
10 μM Nuc_R	1
5X Phusion GC Buffer	4
Phusion DNA Polymerase	0.2
2 mM dNTP mix	2
Template DNA	1
DMSO	0.6
ddH_2O	10.2

Table 6: Thermocycling conditions with two different annealing temperatures.

Step	Temperature [°C]	Time [sec]
Initial Denaturation	98	30
30 x	98	10
	55 (57)	30
	72	60
Final Extension	72	600
Hold	4	N/A

Results and Conclusions

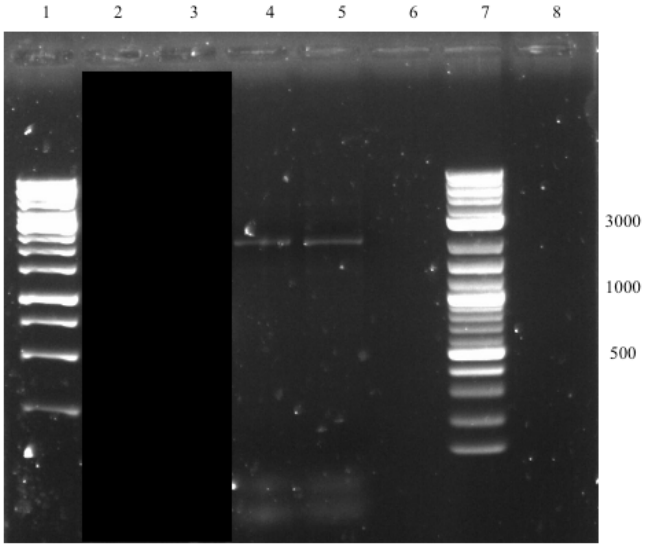

Figure 2: Result of the gel electrophoresis of overhang PCR product, with 1 % agarose at 110 V. (1) DNA Ladder (4) Nuc 55 °C (5) Nuc 57 °(7) DNA Ladder.

Table 7: Concentration of PCR purified PCR product, measured with NanoDrop at 280 nm.

Sample	Concentration [ng/ml]
Nuc 55	9.6
Nuc 57	5.5

Discussion and Troubleshooting

The gel shows faint bands just below the expected size of 2672 bp. The much smaller bands below the ladder corresponds to primer dimers and non-annealed primers. To increase the PCR efficiency and decrease the amount of non-annealed primers, the extension time will be increased from 75 to 120 for the next overhang PCR. After discussion with our supervisor Johan Rockberg, attempts of increasing the annealing temperature according to the NEB T_m Calculator, will be performed.

The Nuc samples were PCR purified since they might the correct product, but since the concentrations of the purification products were too low they were not used for further experiments.